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Research Paper

Evaluation of necrotumic factor, fasting diabetes, fasting insulin and insulin resistance levels in women with gestational diabetes

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ABSTRACT

This study aims to examine the concentrations of tumor necrosis factor-α, endothelial growth factors, fasting insulin, fasting blood glucose, and insulin resistance, in 70 women with gestational diabetes in the city of Kirkuk for the period between November 2022 and April 2023 in Kirkuk General Hospital and the medical and specialized clinics. The age of these women ranges between 20-40 years who were divided into two age groups: (20-29) and (30-40). Each group included (35). In addition, the control group included 20 healthy people who did not suffer from any symptoms. After obtaining blood serum, physiological and biochemical tests were conducted. The results show a significant increase ($p \leq 0.01$) in the concentration of tumor necrosis factor-α (TNF-α), in the level of fasting insulin, fasting blood sugar, and insulin resistance in women with gestational diabetes when compared with the control group. However, both groups of patients (20-29) and (30-40) showed no significant difference in the variables of the study.

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1. INTRODUCTION

Gestational diabetes mellitus (GDM) is one of the types of diabetes that was first diagnosed in the second trimester of pregnancy. It is one of the most complicating conditions during pregnancy worldwide, and affects the mother and fetus. [1] However, it may disappear after childbirth or may develop into type

II sugar. It occurs when beta cells in the pancreas are unable to make enough insulin, especially in the second and third trimesters of pregnancy to maintain healthy blood sugar levels. Furthermore, GDM has become one of the major health problems that has dramatically increased the world healthcare burden. [2] This causes many problems because diabetes increases the risk of complications during pregnancy. In addition, it increases the risk of developing diabetes in the future newborn. Yet, the pregnant mother recovers from GDM as soon as the child is born, but the first and second types accompany the patient. [3] Tumor Necrosis Factor-α (TNF) plays an important role in autoimmune diseases, acute and chronic bacterial and viral infections. Women with GDM with gestational diabetes showed an elevated level of tumor necrosis factor-α -α than their uninfected counterparts. [4] These affected women are at the risk of serious complications during pregnancy, including a significant rise in blood pressure and an increased risk of premature birth congenital malformations in the fetus. GDM occurs due to the failure of the pancreas to produce sufficient insulin as a result of dysfunction of beta cells (β-cells) or that the insulin receptors in skeletal muscle cells and fat cells do not respond to insulin. In either case, glucose is unable to enter the cells and accumulates in the blood. Also, the levels of glucose secretion increases in the urine. [5] Insulin resistance refers to the body's inability to use insulin effectively to lower blood sugar levels, as insulin resistance in GDM patients can lead to high blood glucose levels. This increase in the blood glucose may cause complications for both mother and child, as an increase in insulin resistance occurs due to hormones secreted by the placenta, which reduce sensitivity to insulin in the body [6].

2. METHODOLOGY

Research Designs

This study was conducted in the city of Kirkuk for the period between November 2022 and April 2023. The study included 90 women, 70 GDM pregnant women aged (20-40) years of gestational diabetes after being diagnosed by specialist female doctor and (20) healthy pregnant women who are not (gestational diabetes). All the pregnant women were in the second trimester of pregnancy and the beginning of the last trimester of pregnancy (24-48) weeks from the beginning of pregnancy. They were divided into two groups according to age: (20-29) and (30-40). Then blood samples were collected from patients and healthy people in Kirkuk General Hospital, Azadi Teaching Hospital, Middle East Medical Laboratory, Al-Shafi Laboratory and Al-Methali Specialized Laboratory for pathological analyzes and hormones. All samples underwent laboratory tests examining the concentrations of the tumor necrosis factor-α TNF-α, fasting insulin, fasting sugar, and insulin resistance, in the blood serum. Also, all participants were divided for a comprehensive clinical examination. Then, the patients and the healthy people were divided into 4 groups based on age:

1. The first group of (35) patients (20-29).
2. The second group of (10) normal healthy people (20-29).
3. The third group of (35) patients (30-40).
4. The fourth group of (10) normal healthy people (30-40).

Blood Samples

Blood samples were obtained from women with gestational diabetes at a rate of 5 ml. The blood was placed in the Tub Gel test tube and left for 5/ minutes at room temperature. Then the samples were separated by a centrifuge for 15 minutes at 3000 rpm to obtain blood serum. After that, the blood serum was separated and withdrawn in the Eppendorf tubes and kept at (-20) m until the required tests were performed.

Biochemical Analysis

The basic principle to estimating the concentrations of tumor necrosis factor-α, fasting insulin, fasting sugar, and insulin resistance coefficient is the use of the analysis kit from the French company Biolab. It adopts the most updated enzyme-linked immunosorbent assay (ELISA) techniques.

Statistical Analysis

Statistical analyses were conducted using the ready-made statistical program (SPSS) Statistical package for social sciences V 2. The means were extracted and the T-test was used to compare the means of independent samples (independent samples T-test). In addition, the means the paired samples T-test and the difference were considered significant at the level of p≤0.01), and the immunological and biochemical test values were described as Mean ± standard deviation [7].

3. RESULTS AND DISCUSSION

Concentration of Tumor Necrosis Factor-A in the Blood

The results of the current study in indicate a significant increase (p≤0.01) in the concentration of tumor necrosis factor-α in women with GDM, which reached (0.78±0.07) compared to the control group, which amounted to (0.38±0.05) ng/ml. The two patient age groups were compared with the control. The results of this comparison show a significant increase (0.77±0.09) ng/ml in the (20-29 year) age group compared to the control group, which amounted to (0.36±0.04) ng/ml. Also, the age group (30-40) years was (0.79±0.05) ng/ml whereas the control group rose to (0.39±0.06) ng/ml. However, the comparison of the two groups of patients, the results show that the two age groups were similar. Concentration of Alpha Tumor Necrotic Factor TNF-α in Patients' Serum and Control.

Values expressed in SD ±

- Different capital letters mean there is a significant difference (p≤0.01) between patients and the control and vice versa.
- Different lowercase letters mean that there is a significant difference (p≤0.01) between the age groups of patients and vice versa.

The results of the current study are consistent with the findings of other studies [8], [9], [10], [11] which indicated a high concentration of tumor necrosis factor in women with GDM. This means that there is a positive relationship between GDM and the concentration of alpha tumor necrosis factor. The alpha-tumor necrosis factor TNF-α increased in pregnant women because of its positive and strong association with glycated hemoglobin and insulin resistance as it interferes with the auto phosphorylation of insulin receptors insulin receptor substrate (IRS-1). This interference weakens its binding to insulin receptors [12]. This is because GDM is associated with insulin resistance and poor insulin secretion, and there is an insulin deficiency during pregnancy with high insulin needs to compensate for insulin resistance that develops during the last trimester of pregnancy. In addition, the absorption of glucose in the tissues of the mother tissue depends on insulin and the absorption decreases due to insulin deficiency and hyperglycemia develops after meal. Because the mother-to-placenta glucose transport depends on concentration, maternal hyperglycemia increases glucose transmission from the placenta to the fetus [13].

Fasting Insulin Concentration in Blood Serum

A significant increase (p≤0.01) in the means of fasting insulin concentration among women with GDM, which was (11.65 ±1.7) mmol/L when compared with the control group which reached (4.2 ±0.77) mmol/L. The results show a significant increase in the age group (20-29) years, which was (11.22 ±1.5) mmol/l compared to the control group which amounted to (4.28±0.80) mmol/l. Also, the age group (30-40) years was (12.08 ±2.00) mmol/l while the control group achieved (4.17 ±0.78) mmol/L. However, when comparing the two age groups of patients, no significant differences between them appear. Fasting insulin concentration in patients' blood serum and control.

Values expressed in SD ±

- Different capital letters mean there is a significant difference (p≤0.01) between the groups of patients and the control group and vice versa.
- Different lowercase letters mean that there is a significant difference (p≤0.01) between the age groups of patients and vice versa.

The results of our current study are consistent with those of some scholars [14], [15], [16]. Beta cells must secrete more insulin to maintain blood sugar against insulin resistance. In the development of type 2 diabetes tests, hyperinsulemia with increased blood sugar precedes the development of

hyperglycemia so fasting insulin can be considered as a sign of insulin resistance [15]. Some studies show that fasting insulin rises in women who later develop GDM [16]. The pattern of insulin requirements during pregnancy varies between women with type 1 and type 2 diabetes, indicating a different effect of pregnancy-mediated insulin resistance. Women with type 2 diabetes require a greater increase in insulin dose from the beginning of every trimester and insulin requirements do not decrease at the beginning and end of pregnancy as in women with type 1 diabetes [17].

Fasting Blood Sugar Concentration in Blood Serum

The results of the current study in a significant increase (p≤0.01) in the means of fasting blood sugar concentration among women with GDM, which amounted to (7.40±1.18) mmol/L when compared with the control group (4.61 ±0.79) mmol/L. When each group of patients is compared with the control group, the results show a significant increase in the age group (20-29) years, which amounted to (7.59 ±1.16) mmol/L, compared to the control group (5.14 ±0.65) mmol/L. The age group (30-40) years was (7.20 ±1.18) mmol/L while the control group was (4.08 ±0.50) mmol/L. However, the comparison of two age groups of patients shows no significant difference between them. Fasting sugar concentration in patients' blood serum and control.

Values expressed in SD ±

- Different capital letters mean there is a significant difference (p≤0.01) between the groups of patients and the control groups and vice versa.
- Different lowercase letters mean that there is a significant difference (p≤0.01) between the two age groups of patients and vice versa.

These results agree with other research works [16], [17], [18] reporting elevated fasting blood sugar concentrations in women with GDM. Pregnant women develop postprandial glycemic responses as growth progresses, unlike fasting glucose levels in which glucose increases after pregnancy by 27-43 mg/100 ml between the first and third trimesters of pregnancy [16].

Insulin Resistance

According to the results of the current study, the means of insulin resistance) p≤0.01) significantly increased among women with GDM to (3.83±2.04) in relation to the control group, which reached (0.87±0.19). Also, the age group (20-29) significantly rose to (3.78±1.64) compared to the control group, which amounted to (0.98±0.14) while the age group (30-40) years was (3.87±2.31) in comparison to the control group, which was (0.76±0.19). However, the two age groups of patients reveal no significant difference between them. Patient Serum Sulin Resistance Concentration and Control.

Values expressed in SD ±

- Different capital letters mean there is a significant difference (p≤0.01) between patients and control and vice versa.
- Different lowercase letters mean that there is a significant difference (p≤0.01) between the age groups of patients and vice versa.

These results agree with some other studies [19], [20], [21], [22]. These studies reported increased insulin resistance in pregnant women. Insulin resistance changes over time during pregnancy, and in the latter half of pregnancy insulin resistance increases significantly and can become severe, especially in women with GDM and type 2 diabetes. Many factors such as placental hormones, obesity, lack of activity, unhealthy diet, and genetic and genetic contributions affect insulin resistance during pregnancy [21]. Insulin resistance is the biological response to a certain internal or external dose of insulin in target tissues (liver, muscle or adipose tissue) [23]. In normal pregnancy, the mother's tissues become increasingly insulin insensitive as a 50-60% reduction in insulin sensitivity has been observed with pregnancy progression in both women with normal glucose tolerance and women with GDM. However, in women with normal glucose tolerance changes in insulin sensitivity are overcome through the increase in insulin production by pancreatic beta cells. Yet, in women with diabetes insulin secretion during pregnancy is insufficient [20]. Increased insulin resistance in skeletal muscles of the mother is an essential feature of GDM patients, which is responsible for increasing the supply of nutrients to the fetus and ultimately enhances fetal obesity. Studies have shown that the pathway of insulin signaling and glucose absorption in

skeletal muscles from pregnant women are affected largely due to pro-inflammatory TNF-α cytokines [24], [25].

4. CONCLUSION

According to the results, the concentration of tumor necrosis factor-α TNF-α and the concentration of fasting insulin, fasting sugar and insulin resistance increased.

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Authors Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Sarah Jamal Jameel	✓	✓	✓		✓	✓		✓	✓	✓	✓		✓	✓
Sahib J. Abdulrahman	✓	✓		✓			✓		✓	✓		✓	✓	
Sabah Hussain Khurshid	✓	✓		✓			✓		✓	✓		✓	✓	

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Informed Consent

All participants were informed about the purpose of the study, and their voluntary consent was obtained prior to data collection.

Ethical Approval

The study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki and approved by the relevant institutional authorities.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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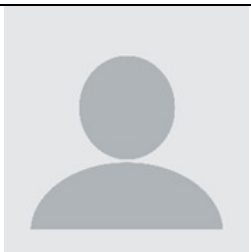
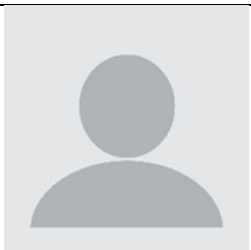
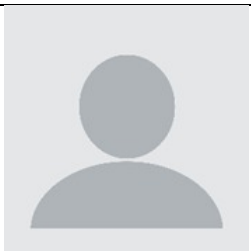
	**Sarah Jamal Jameel,** is a master's student at the College of Education for Pure Sciences, University of Kirkuk, Iraq. Her research focuses on biochemical and physiological aspects of metabolic disorders, particularly gestational diabetes mellitus. She has a strong interest in studying hormonal and inflammatory biomarkers related to insulin resistance and glucose metabolism in women's health. Email: jamalsara213@gmail.com
	**Prof. Sahib J. Abdulrahman,** is a professor at the College of Education for Pure Sciences, University of Kirkuk, Iraq. His expertise lies in medical biochemistry and endocrinology, with extensive experience in supervising postgraduate research on metabolic and hormonal disorders. Email: drsahib68@uokirkuk.edu.iq
	**Prof. Sabah Hussain Khurshid,** is a professor at the College of Education for Pure Sciences, University of Kirkuk, Iraq. His research interests include molecular biology, clinical biochemistry, and the physiological mechanisms underlying metabolic diseases such as diabetes and obesity. Email: Sabahkorsheed49@gmail.com